Alterations in Growth, Photosynthesis, and Respiration in a Starchless Mutant of *Arabidopsis thaliana* (L.) Deficient in Chloroplast Phosphoglucomutase Activity[1]

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ABSTRACT

A mutant of *Arabidopsis thaliana* (L.) Heynh. which lacks leaf starch was isolated by screening for plants which did not stain with iodine. The starchless phenotype, confirmed by quantitative enzymic analysis, is caused by a single recessive nuclear mutation which results in a deficiency of the chloroplast isozyme of phosphoglucomutase. When grown in a 12-h photoperiod, leaves of the wild-type accumulated substantial amounts of starch but lower levels of soluble sugars. Under these conditions, the mutant accumulated relatively high levels of soluble sugars. Rates of growth and net photosynthesis of the mutant and wild-type were indistinguishable when the plants were grown in constant illumination. However, in a short photoperiod, the growth of the mutant was severely impaired, the rate of photosynthesis was depressed relative to the wild-type, and the rate of dark respiration, which was high following the onset of darkness, exhibited an uncharacteristic decay throughout the dark period. The altered control of respiration by the mutant, which may be related to the relatively high levels of soluble carbohydrate that accumulate in the leaf and stem tissue, is believed to be partially responsible for the low growth rate of the mutant in short days. The depressed photosynthetic capacity of the mutant may also reflect a metabolic adaptation to the accumulation of high levels of soluble carbohydrate which mimics the effects of alterations in source/sink ratio. The activities of sucrose phosphate synthase and acid invertase are significantly higher in the mutant than in the wild-type whereas ADP-glucose pyrophosphorylase activity is lower. This suggests that the activities of these enzymes may be modulated in response to metabolite concentrations or flux through the pathways.

The accumulation of nonstructural carbohydrate in leaves has been suggested to influence both the rate of photosynthesis and the rate of dark respiration. An effect of leaf carbohydrate concentration on photosynthesis has been repeatedly proposed as a possible explanation for the depression of photosynthetic rate which may result from experimental treatments which increase the source/sink ratio or decrease the rate of translocation

of photosynthate from a source leaf (1, 11, 18). However, interpretation of the results of such experiments has been complicated by the possibility that hormonal or other changes may result from the treatment, and from instances in which no correlation has been observed between leaf carbohydrate concentration and photosynthesis rate (17, 20, 22). An inherent problem in attempting to establish explanatory correlations is that most experimental manipulations do not permit control of the molecular species of carbohydrate which accumulates. Thus, potential regulatory effects of sucrose or other sugars may be obscured by mechanisms which regulate starch/sucrose partitioning and prevent soluble carbohydrate from accumulating (12, 23, 28).

The effect of carbohydrate accumulation on the rate of dark respiration has received less attention but appears to have been consistently observed by a variety of approaches (2, 6, 29). The essential observation is that the rate of respiration is proportional to carbohydrate content or is stimulated by provision of exogenous carbohydrate. The implication is that the amount of respiration is regulated by substrate supply rather than demand for ATP or reducing equivalents. Indeed, it has been suggested that, under conditions of high carbohydrate supply, a substantial proportion of the reductant generated during carbohydrate catabolism may be consumed by the alternative oxidase without being linked to ATP production (3, 15). Thus, such respiration may be considered as potentially wasteful and may represent a target for genetic manipulation.

To investigate the role of starch in leaf carbohydrate metabolism, we have isolated several mutants of *Arabidopsis thaliana* which are unable to synthesize leaf starch. We were specifically interested in observing to what extent photosynthate would accumulate in the mutants as soluble sugars rather than starch, and what effects this might have on photosynthesis, respiration, and growth. We describe here the properties of one such mutant which is unable to convert glucose-6-P to glucose-1-P in the chloroplast, because of a deficiency of the chloroplast isozyme of PGM[2], and hence unable to synthesize starch.

MATERIALS AND METHODS

Plant Material and Growth Conditions. The mutant lines TC7, TC9, and TC135 were isolated from the Columbia wild-type of *Arabidopsis thaliana* (L.) Heynh. following mutagenesis with ethyl methane sulfonate by previously described procedures (26). The mutant lines, maintained as homozygotes by self-fertilization, were advanced for six to eight generations before being used

[2] Abbreviations: PGM, phosphoglucomutase; PGI, phosphoglucoisomerase; SPS, sucrose phosphate synthase.

for the physiological experiments reported here. Unless otherwise indicated, plants were grown at approximately 22°C with cool-white fluorescent illumination (200 μE m^{-2} s^{-1}) on a per-lite:vermiculite:sphagnum (1:1:1) mixture irrigated with a mineral nutrient solution (26).

Starch Gel Electrophoresis. Leaf material was ground with an equal weight of buffer containing 100 mM Tris·Cl (pH 7.5), 100 mM KCl, 100 mM sucrose, 40 mM 2-mercaptoethanol, and 5 mM EDTA. The crude extract was absorbed by a small strip of Whatman 3MM paper which was inserted into a 12% (w/v) starch gel in 50 mM Tris·borate (pH 8.0) and 1.6 mM EDTA (24). The sample was electrophoresed at 6 v/cm for 9 h at 4°C, then the gel was sliced horizontally into 1-mm slabs and stained for PGM and PGI activity (24). The stained gels were fixed with ethanol:acetic acid:glycerine:water (5:2:1:4) and photographed.

The chloroplast specific isozymes were identified by first purifying isolated intact chloroplasts from protoplasts (25). The chloroplasts were ruptured by osmotic shock and the extract was subjected to electrophoresis as described above for whole leaves.

Gas Exchange Measurements. Methods for short-term gas exchange measurements on single intact plants have been described (26). For long-term gas exchange measurements, a Plexiglas chamber was constructed to snugly hold a 13-cm pot with a 4.5-cm head space above the pot. Gas inlets and outlets were designed to direct the gas stream across the surface of the plants so as to minimize the boundary layer resistance of intact plants growing in the pots. The CO_2 concentration in the entering and exiting gas stream was continuously monitored with an Analytical Development Company Series-225 IR gas analyzer in differential mode. The irradiance was 400 μE m^{-2} s^{-1} (PAR). Measurements of photosynthesis and respiration were corrected for exchange due to the nonleaf material in the pot at the completion of an experiment by removing all leaf material and repeating the gas exchange measurements on the harvested pot. Chl was determined in ethanol (30).

Carbohydrate Measurements. For genetic screening, the presence of starch (amylose) in leaves was qualitatively determined by staining for 30 min with a solution containing 5.7 mM iodine, 43.4 mM potassium iodide in 0.2 N HCl. Leaves were decolorized before staining by soaking in 96% ethanol for about 6 h. Quantitative measurements of leaf carbohydrate were performed by homogenizing leaf samples in 80% ethanol. Starch was estimated as glucose released by amyloglucosidase treatment of the ethanol insoluble fraction (23). The ethanol soluble fraction was evaporated to dryness and then the residues were resuspended in water and assayed for hexose and sucrose content (23).

Enzyme Assays. Extracts were prepared by grinding leaf samples in cold 50 mM sodium Hepes (pH 7.5), 5 mM MgCl₂, 1.0 mM EDTA, 2.5 mM DTT, 2% PEG-20 (w/v), and 1% BSA (w/v). Insoluble matter was removed by centrifugation at 38,000g for 10 min and an aliquot of the extract was desalted by passage through a small column of Sephadex G-25. Assay procedures for SPS (23), cytoplasmic fructose bisphosphatase (23), starch synthase (5), UDP-glucose pyrophosphorylase (27), ADP-glucose pyrophosphorylase (27), and invertase (13) have been described.

Measurement of Growth Rate. Plants were grown as described above except the irradiance was 500 μE m^{-2} s^{-1}. At various times (noted in the text), samples of five plants of each genotype were harvested for fresh weight determinations.

RESULTS

Mutant Isolation. The mutant isolation protocol was based on the assumption that the absence of leaf starch would impose no deleterious effect on plants growing in continuous illumination. In the first attempt at mutant isolation, leaves were removed from approximately 1500 M2 plants descended from ethyl methane sulfonate mutagenized seed, and stained for the presence of

starch with iodine. Two starchless plants from this population gave rise to lines (designated TC7 and TC135) which have remained uniformly starchless for the six generations tested so far. In a subsequent screen with an independently mutagenized batch of seed, one starchless mutant line (TC9) was recovered from among approximately 700 M2 plants by the same method. Except for the starchless character, the mutants are phenotypically indistinguishable from the wild-type when grown in continuous illumination.

A quantitative measurement of the starch content of the mutant line TC7 and the wild-type was obtained by measuring the ethanol-insoluble carbohydrate concentration of plants grown in a 12-h light/12-h dark photoperiod (Fig. 1) or in continuous illumination (Table I). The leaves of the mutant were almost completely lacking in starch under all conditions. At the level of detection of the iodine stain, the mutants completely lacked starch in leaf, stem, and root tissue.

Biochemical Characterization. The diversion of carbon from the Calvin cycle to amylose involves only four enzymic steps: PGM, PGI, ADP-glucose pyrophosphorylase, and starch synthase (21). Thus, the strategy for determining the biochemical basis for the starchless phenotype was straightforward except for the fact that PGM and PGI exist as several isozymes in all plants
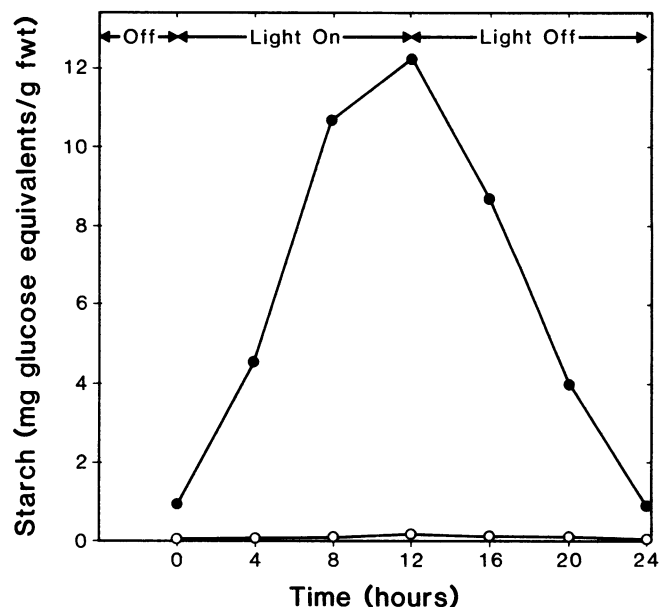

FIG. 1. Diurnal changes in starch concentration in leaves of wild-type (●) and mutant (○) A. thaliana. The plants were grown in a 12-h photoperiod. The wild-type plants were 28 to 35 d old and the mutant plants were 49 to 56 d old. Symbols represent the means of measurements made on two samples of leaves. The data points at 0 and 24 h represent the same samples which are repeated simply for clarity.

Table I. *Carbohydrate Concentrations in Wild-Type and Mutant A. thaliana*

Plants were grown in continuous illumination. Values represent the mean of two measurements.

Strain	Atmosphere	Carbohydrate Concentration	
		Starch	Soluble sugars
		mg glucose equivalents g⁻¹ fresh wt	
Wild-type	Ambient	7.70	1.04
TC7	Ambient	0.25	1.13
Wild-type	1% (v/v) CO₂	78.30	2.54

examined (10). The presence of multiple isozymes in leaf tissue can complicate interpretation of activity measurements made on crude extracts. Therefore, we examined the activity of PGM and PGI isozymes by histochemical localization following resolution of the isozymes by electrophoresis in starch gels. The results of these experiments (Fig. 2) revealed three PGM isozymes and two PGI isozymes in the wild-type. As a matter of convenience, the three isozymes have been designated PGM^f (fast), PGM^i (intermediate), and PGM^s (slow). The fast isozyme of both PGI and PGM is the chloroplast isozyme since this is the only activity which was recovered from isolated intact chloroplasts from the wild-type (Fig. 2). The presence of three isozymes of PGM in *A. thaliana* is unusual because most plant species have only two (10). This may represent a case of a single locus gene duplication as has been suggested for anomalous isozyme patterns in other species (10).

The mutant line TC7 had both of the PGI isozymes found in the wild-type (Fig. 2), normal levels of starch synthase (Table II), about one-third as much activity of ADP-glucose pyrophosphorylase as the wild-type (Table II), but completely lacked activity of the chloroplast isozyme of PGM (Fig. 2). Since the mutant appears to have adequate levels of the other enzymes required for starch biosynthesis, we consider it extremely likely that the loss of chloroplast PGM activity is the biochemical basis for the starchless phenotype in this mutant. The reason for the reduced activity of ADP-glucose pyrophosphorylase is not known but may reflect a regulated response to the absence of flux through the starch biosynthetic pathway. Subsequent analysis of the other
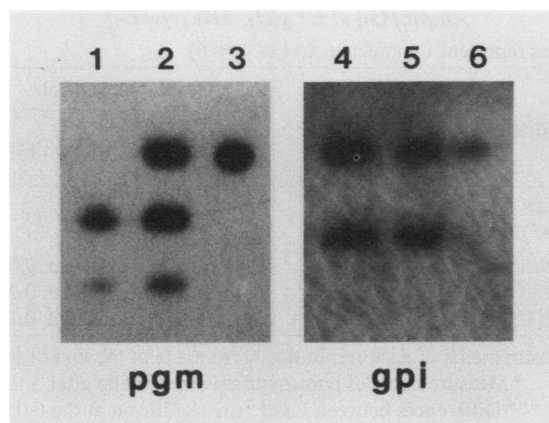

FIG. 2. PGM and PGI isozymes in the starchless mutant TC7 and wild-type *A. thaliana*. Whole leaf extracts from the mutant (lanes 1 and 4) and wild-type (lanes 2 and 5) or from isolated intact wild-type chloroplasts (lanes 3 and 6) were electrophoresed in a starch gel which was then sliced and stained for activity. The direction of migration was toward the anode at the top.

Table II. *Effect of the pgmP Mutation on the Activity of Certain Enzymes Associated with Starch and Sucrose Metabolism in Leaves*

Plants were grown in a 12-h photoperiod and harvested at the end of the light period. Values represent the mean ± SE ($n = 3$).

Enzyme	Specific Activity	
	Wild-type	Mutant (TC7)
	$\mu mol\ g^{-1}\ fresh\ wt\ h^{-1}$	
ADP-glucose pyrophosphorylase	69 ± 3	23 ± 1
Starch synthase (soluble)	7.7 ± 1.4	10.2 ± 1.0
Fructose bisphosphatase[a]	103 ± 10	108 ± 15
UDP-glucose pyrophosphorylase	525 ± 20	663 ± 25
Acid invertase	99 ± 19	212 ± 16

[a] Cytoplasmic enzyme.

starchless mutant lines may be useful in resolving the basis for this effect.

Genetic Characterization. The genetic basis for the starchless phenotype was established by determining the presence of starch in the leaves of the F1 and F2 progeny from a cross between the wild-type and the mutant line TC7. The F1 progeny from this cross had leaf starch and had all three isozymes of PGM. Of 84 F2 progeny from this cross, 23 lacked starch. This satisfactory fit ($\chi^2 = 0.25$; P > 0.5) to the 3:1 hypothesis, and the properties of the F1 hybrid are entirely consistent with the presence of a single nuclear recessive mutation. We have designated the locus defined by this mutation *pgmP* (*i.e.* the plastid isozyme of PGM).

To substantiate the relationship between the loss of the PGM^f isozyme and the starchless phenotype, 54 F2 plants from a wild-type × TC7 cross were tested both for starch and for the presence of the PGM^f isozyme. All of the 14 plants that lacked starch also lacked activity of the PGM^f isozyme. All other plants contained both starch and the PGM^f isozyme. This cosegregation of the starchless phenotype and the absence of the PGM^f isozyme makes it very unlikely that two separate biochemical deficiencies are responsible for the starchless phenotype.

We have not, as yet, examined the properties of the other two starchless mutants in detail. However, genetic complementation studies suggest that the starchless phenotype of one of the other lines (TC9) is also due to a mutation in *pgmP* since the F1 plants resulting from the TC7 × TC9 cross lacked starch. In contrast, the F1 hybrids obtained by crossing the starchless line TC135 with TC7 or TC9 all had normal levels of starch. Thus, the mutant line TC135 carries a lesion at another locus.

Measurement of Growth. To quantitate the effects of the starchless phenotype on growth rate, the rate of increase in fresh weight of mutant and wild-type plants was measured for plants growing in various photoperiods. The results of this experiment (Fig. 3) show that when plants were grown in continuous illumination, the growth rate of the mutant was indistinguishable from that of the wild-type. However, as the length of the diurnal dark period was increased, growth of the mutant was differentially impaired relative to that of the wild-type. Thus, for example, after 37 d of growth on a 7-h light/17-h dark photoperiod, the fresh weight of the mutant was only 10% that of the wild-type (Fig. 3).

The similarity of the growth rate of mutant and wild-type in continuous illumination indicates that the *pgmP* mutation is only conditionally deleterious. A requirement for a long photoperiod has also been observed in the other starchless mutants (results not presented), suggesting that the effect is specifically
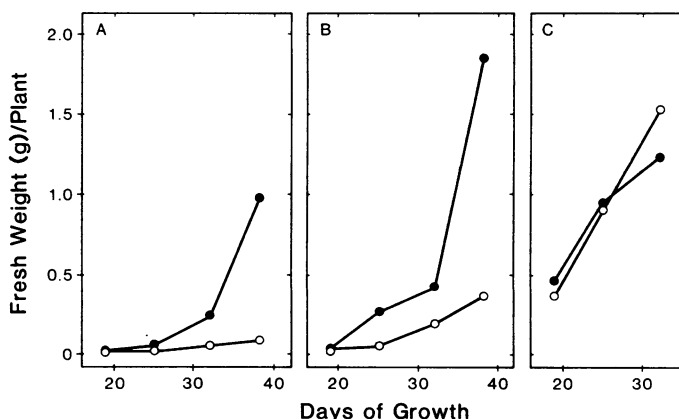

FIG. 3. Effect of photoperiod on growth of mutant (O) and wild-type (●) *A. thaliana*. The daily period of illumination was: (A), 7 h, (B), 12 h, (C), 24 h. Symbols are means of measurements made on two or three samples.

related to the absence of starch *per se*. The indistinguishable growth rate of mutant and wild-type in continuous light also indicates that there are no other significant deleterious genetic defects in the background genotype of the line TC7.

Diurnal Changes in Carbohydrate. To provide a basis for interpreting the gas exchange and growth characteristics of the mutant, the nonstructural carbohydrate concentrations of leaves were determined at various times during a light/dark cycle (12-h photoperiod). The results of this experiment showed that the wild-type accumulated substantial amounts of starch (Fig. 1) but very little soluble carbohydrate (Fig. 4). By contrast, quantitative analysis of the carbohydrate concentration of the mutant confirmed that it had barely detectable levels of starch at all times of the day, but that photosynthate accumulated in the mutant in the form of sucrose and hexose sugars (equal amounts of glucose and fructose). Carbohydrate also accumulated in the stems of both wild-type and mutant plants to about one-half the concentration found in leaves. In the wild-type, this consisted almost entirely (88%) of starch. No starch was detectable in the stem tissue of the mutant and the accumulated carbohydrate was almost exclusively (98%) hexose sugars.

Respiratory and Photosynthetic Characteristics. To measure gas exchange of plants growing on a day/night cycle, consideration must be given to the possibility of diurnal fluctuations in photosynthetic and respiratory characteristics. This was considered particularly important in the case of the mutants because of the unknown effects of the atypical accumulation of soluble sugars throughout the period of illumination (Fig. 4).

The effect of time-of-day on gas exchange characteristics was determined by continuous measurements of a population of intact plants throughout the day/night cycle. Our experience suggests that this experimental approach is not optimal for obtaining highly accurate measurements of gas exchange rates because of background gas exchange due to the roots and microorganisms in the potting mixture. Nevertheless, the method appeared to be satisfactory for observing major changes in the rate of gas exchange. The results of this experiment (Fig. 5) indicated that the photosynthetic rate is essentially stable throughout the 12-h photoperiod used for this experiment in both mutant and wild-type. Because of the observation that soluble carbohydrate accumulation increases 10-fold in the mutant throughout the photoperiod (Fig. 4), it is apparent that this accumulation of soluble sugars had no immediate deleterious effect on photosynthetic rate. Similarly, the accumulation of starch in the wild-type also had no effect on photosynthesis. More accurate short-term measurements of the photosynthesis rate of plants grown in a 12-h photoperiod confirmed that the
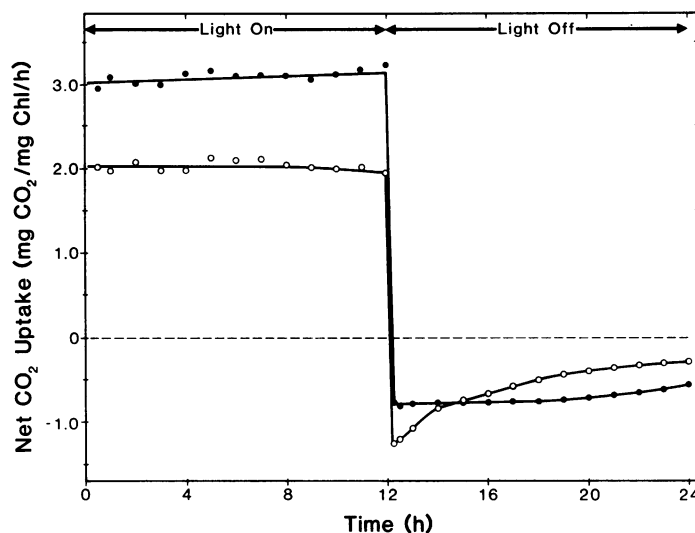
FIG. 5. Net CO_2 uptake by wild-type (●) and mutant (○) *A. thaliana*. Plants were grown in a 12-h photoperiod. The wild-type plants were 24 to 26 d old and the mutant plants were 30 to 48 d old. The symbols represent the average value obtained in three independent experiments in the case of the mutant and two in the case of the wild-type. The mutant and wild-type leaves contained the same amount of Chl (2.4 mg Chl dm^{-2}).

Table III. *Gas Exchange Rates for Mutant and Wild-Type A. thaliana in Air (350 μl L^{-1} CO_2, 21% [v/v] O_2)*

Values represent the mean ± SD (3≤ n ≤ 6).

Irradiance	Growth Photoperiod (Light/ Dark)	Gas Exchange Rate	
		Wild-type	Mutant (TC7)
μE $m^{-2}s^{-1}$	h	mg CO_2 mg^{-1} Chl h^{-1}	
Darkness[a]	12/12	0.45 ± 0.08	0.53 ± 0.08
180[b]	12/12	3.48 ± 0.31[c]	2.84 ± 0.21[d]
200	24/0	2.61 ± 0.31[d]	2.49 ± 0.15[d]

[a] Measurements of dark respiration were made at the end of the dark cycle. [b] Measurements of photosynthesis were made after 5 to 7 h of light. [c,d] Differences between [c] and [d] are significant at the 0.01 level.

mutant had reduced photosynthetic capacity relative to the wild-type (Table III). This indicates a long-term effect of the *pgmP* mutation on photosynthetic capacity.

The respiratory response of the mutant was qualitatively and quantitatively different from that of the wild-type (Fig. 5). Whereas the rate of respiration of the wild-type was almost constant throughout the dark period, the mutant had a relatively high rate of respiration at the onset of the dark period, which then decreased in parallel with the concentration of soluble sugars (Fig. 4). More accurate short-term measurements of the respiration rate at the onset of darkness confirmed the significant difference between the respiration rate of the mutant (0.793 mg CO_2 mg^{-1} Chl h^{-1}) and the wild-type (0.437 mg CO_2 mg^{-1} Chl h^{-1}). Similarly, short-term measurements of the respiration rate at the end of the 12-h dark period (Table III) indicated that wild-type and mutant had comparable respiratory rates at this time. If these values are used to normalize the long-term measurements of respiration, it appears that the total amount of respiratory CO_2 evolved by the mutant during the dark period was about 1.5 times greater than the amount evolved by the wild-type.

Effects of Growth in Continuous Illumination. In an attempt to determine the basis for the effect of photoperiod on the growth
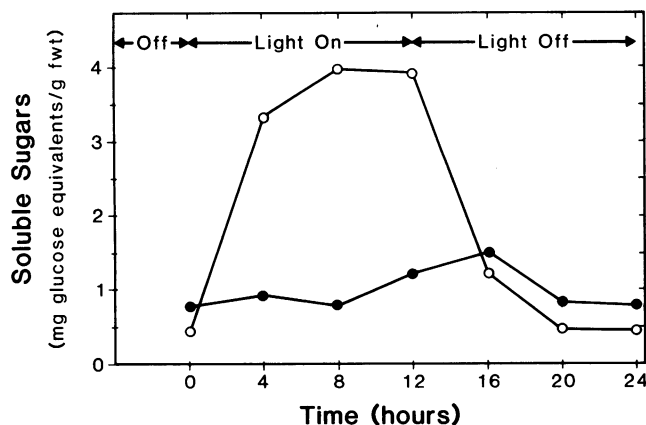

FIG. 4. Diurnal changes in soluble carbohydrate concentration in leaves of wild-type (●) and mutant (○) *A. thaliana*. Conditions were the same as in Figure 1.

of the mutant, we conducted a preliminary analysis of the carbohydrate metabolism of plants grown in continuous light. In these conditions, wild-type and mutant plants accumulated the same concentration of soluble sugars (Table I). Somewhat surprisingly, both mutant and wild-type plants contained less carbohydrate than the maximal level accumulated by the same lines when grown in a 12-h light/dark cycle. It is not known what factors regulate the steady-state level of carbohydrate in the plants grown in continuous light. However, it is interesting to note that when the wild-type was grown in an atmosphere enriched with 1% (v/v) CO_2, it accumulated 10-fold more starch than when grown in air under otherwise identical conditions (Table I). The implication is that in standard atmospheric conditions the amount of starch accumulation is probably not limited by inherent structural constraints.

When grown in continuous illumination, the photosynthesis rate of the wild-type and mutant were indistinguishable (Fig. 6) and, as noted above, the two lines had indistinguishable growth rates (Fig. 3). Considered in conjunction with the foregoing analysis of carbohydrate content, these results suggest that in continuous illumination, the wild-type and mutant are functionally equivalent. However, comparison of photosynthesis measurements made on plants grown in an alternating light/dark cycle and in continuous light indicated that the photosynthetic capacity of the wild-type, but not the mutant, was significantly lower for plants grown in continuous illumination than in a 12-h photoperiod (Table III). The observation that the photosynthetic capacity of the mutant is not affected by photoperiod raises the possibility that the depression of wild-type photosynthetic capacity by growth in continuous light is related to decreased net synthesis of starch. It seems likely that in both the wild-type grown in continuous light, and in the mutant under all conditions, the apparent increase in synthesis of sugars may trigger an adaptation which results in relatively depressed photosynthetic capacity. In this respect, these observations are consistent with the proposal that high levels of soluble carbohydrate may depress photosynthetic capacity (1). However, because there was no apparent reduction of the photosynthetic rate of the mutant throughout the course of a single photoperiod (Fig. 5) in which soluble carbohydrate concentration increased about 10-fold (Fig. 4), the mechanisms must involve relatively long-term metabolic adaptation.

Regulation of Sucrose Phosphate Synthase Activity. There is substantial evidence implicating SPS as a regulatory step in sucrose biosynthesis (7, 12, 23). Because of the greater amount

of sucrose accumulation in the mutant, it was of interest to compare the activity of SPS and related enzymes in the mutant and wild-type. In attempting to resolve some initial difficulties in obtaining reproducible SPS activity measurements on plants grown in different photoperiods, it was found that there was a pronounced effect of photoperiod on SPS activity. In both mutant and wild-type, the amount of activity increased as the duration of the photoperiod increased. However, the effect was less pronounced in the mutant which had levels of SPS activity similar to the wild-type when grown in continuous illumination, but significantly higher levels when grown in a 7-h photoperiod (Fig. 7). These observations are consistent with the evidence, noted earlier, that the wild-type and the mutant are functionally similar in continuous light. The other enzymes of sucrose biosynthesis which were assayed (cytoplasmic fructose bisphosphatase and UDP-glucose pyrophosphorylase) showed no major difference in activity, but invertase activity was 2-fold higher in the mutant (Table II). These observations suggest that SPS and invertase activity may be regulated in response to the amount of flux through the sucrose biosynthetic pathway.

DISCUSSION

In spite of substantial interest in the biochemistry of starch biosynthesis and degradation (21), the effects of starch on photosynthesis (11, 28), stomatal function (19), and gravitropism (14), there have been no previous studies employing a mutant lacking leaf starch. The mutants described here were relatively easy to isolate by direct screening, and mutations at two loci have been isolated. However, it should be noted that *A. thaliana* is an oilseed and probably does not require starch as a seed carbohydrate reserve. Species which rely on seed starch reserves could, in principle, be dependent on the same genes for both leaf and seed starch biosynthesis. Thus, the recovery of mutants lacking leaf starch might not be possible in these species because such mutants would be seedling lethals.

The presence of very low but detectable levels of starch in the mutant line TC7 may be explained in several ways. First, the lesion in the *pgmP* gene in TC7 could be somewhat leaky so that sufficient PGM activity is present in the chloroplast to allow the synthesis of small amounts of starch but not permit visualization of the activity by histochemical techniques (Fig. 2). Second, small amounts of glucose-1-P may enter the chloroplast from the cytoplasm and be incorporated into starch. Third, the amyloglucosidase treatment we used to degrade the starch for quantitation may release small amounts of sugar from polysaccharides
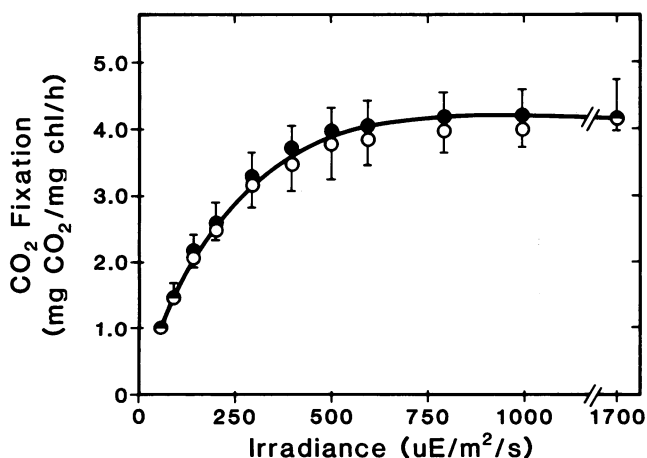

FIG. 6. Light-response curve for photosynthetic CO_2-fixation by wild-type (●) and mutant (○) *A. thaliana*. The plants were grown in continuous illumination (200 μE m^{-2} s^{-1}). Symbols represent the mean ± SD (bars) ($n = 3$).
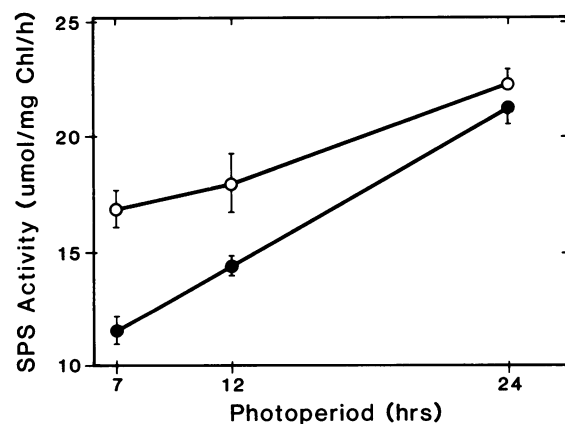

FIG. 7. Effect of photoperiod on activity of sucrose phosphate synthase in wild-type (●) and mutant (○) *A. thaliana*. Plants were grown for 21 d in the indicated photoperiods. Leaves were harvested at the end of the 7- and 12-h photoperiods. Symbols represent the mean ± SE (bars) ($n = 3$).

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other than starch. In this respect, the measured levels are so close to the limit of detection of the methods used (about 0.1 mg g^{-1} fresh weight) that it may simply represent an unavoidable background reaction. Analysis of the other starchless mutants (TC9 and TC135) may be useful in distinguishing among these possibilities.

The presence of three isozymes of PGM in *A. thaliana* is unusual since all other diploid plant species examined to date have only two (10). Two of the PGM isozymes in *A. thaliana* are extrachloroplastic and are presumably involved in cytoplasmic metabolism. It seems possible that the gene encoding the cytoplasmic isozyme has undergone gratuitous gene duplication, as suggested to explain other instances of apparently redundant isozymes (10). Gene duplication seems particularly noteworthy in *A. thaliana* because the unusually small genome size (16) implicitly suggests an economy of organization.

One of the distinguishing characteristics of the mutant is that, because it is unable to store net photosynthate in starch, it accumulates relatively large quantities of sucrose and hexose in both leaf and stem tissue. The accumulation of abnormally high concentrations of soluble carbohydrate *per se* has no obvious short-term effect on photosynthesis since the photosynthetic rate of mutant plants grown in a 12-h photoperiod does not change as carbohydrate accumulates throughout the period of illumination (Fig. 5). However, the photosynthesis rate of the mutant is reduced, relative to the wild-type, when both are grown in short days (Table III). This effect is probably due to one of two major possibilities. First, it may be due to a specific long-term adjustment of photosynthetic capacity brought about in response to the atypical accumulation of soluble sugars. This is, in effect, similar to the hypothesis proposed to explain the depression of photosynthesis which may be observed following sink removal or other experimental treatments which alter consumption of photosynthate by nonsource tissue (18). Second, the reduced photosynthesis rate of the mutant in a 12-h photoperiod may be just one manifestation of a general reduction in metabolic capacity. Such an effect could, for example, be primarily related to the apparent enhancement of respiratory loss associated with the increased accumulation of soluble carbohydrate in the mutant. Although the results presented here do not distinguish between these possibilities, we favor the concept that the accumulation of soluble carbohydrate may trigger a negative long-term regulatory influence on photosynthetic capacity. This could explain why growth in continuous light has no effect on the photosynthetic capacity of the mutant but depresses the photosynthetic capacity of the wild-type to a level comparable to that of the mutant.

The observation that wild-type plants grown in continuous illumination in air accumulated only about one-tenth as much starch as plants grown in air enriched with 1% (v/v) CO_2 (Table I), and only about one-half as much as plants grown in a 12-h photoperiod (Fig. 1) suggests that the net starch synthesis ceases in wild-type plants in continuous illumination not because the size of the starch pool has reached an intrinsic limit, but rather, that an unidentified regulatory mechanism is involved. Furthermore, the steady-state level of total storage carbohydrate in plants of either genotype grown in continuous light is substantially lower than the maximal levels observed during growth in a 12-h photoperiod. We are unable to explain this observation which we believe merits further study. However, it may be related to the intriguing observation that soybeans grown in a 7-h photoperiod partitioned about twice as much photosynthate into starch and sugars as did plants grown in a 14-h photoperiod (4). Finally, it may be worth noting that in continuous light the rate of net CO_2 assimilation and carbohydrate utilization for growth must be equal. Thus, since the mutant has the same photosynthesis rate in both continuous light and a 12-h photoperiod, the rate of utilization of assimilate in the light must be substantially lower for plants grown in a 12-h light/dark cycle.

In addition to the effects on overall photosynthetic rate, a regulatory effect associated with increased flux of carbon through the sucrose biosynthetic pathway is suggested by the observation that SPS activity was increased in response to increased duration of photoperiod in both mutant and wild-type (Fig. 7). This enzyme has recently attracted attention because of an apparent correlation between the amount of extractable SPS activity and the partitioning of photosynthate into starch or sucrose (7, 12, 23, 28). The essential question which has emerged from this correlative approach is whether the SPS activity is the cause or effect of altered partitioning. The results obtained with the mutant do not directly distinguish between the two possibilities. However, it seems apparent from results presented here that SPS activity may be modulated in the long term by substrate availability or the flux of carbon into hexose phosphate. This explains why the mutant has greater SPS activity than the wild-type when grown under a light/dark regime. Presumably, as the duration of the photoperiod is increased, the wild-type partitions an increased proportion of photosynthate into soluble carbohydrate until, as noted earlier, it becomes functionally equivalent to the mutant in continuous light. In these circumstances, the mutant and wild-type apparently have the same amount of flux through the sucrose biosynthetic pathway and the same amount of SPS activity.

One of several interesting characteristics of the *pgmP* mutant is the effect of time-of-day on the respiration rate of the mutant. An analogous effect, observed in wild-type plants of some other species (3), has been interpreted as evidence that the amount of respiration is responsive to the amount or form of storage carbohydrate (3). Because the starchless mutant accumulates high levels of soluble carbohydrate in the light which rapidly decline in the dark, it seems likely that, as suggested (2, 3, 6), the amount of respiration is proportional to the availability of substrate rather than to the demand for ATP and NADH. It remains to be seen if the abnormally high respiration of the mutant is due to alternative oxidase activity which has been invoked to explain substrate-regulation of respiration (3, 15, 29). This concept is of particular interest because of the possibility that crop productivity might be amplified by control of nonproductive respiratory losses (9). In this context, the *pgmP* mutant may afford a means of selecting directly for loss of the wasteful component by selecting for secondary mutations which enhance the growth rate of the *pgmP* mutant in a 7-h photoperiod.

If, as suggested, the instability of the respiratory response indicates that some proportion of respiration is regulated by the amount of substrate rather than demand for ATP (or demand for availability of ADP [8]), then the deleterious effects of the *pgmP* mutation on growth may be partially explained by proposing the existence of a nonproductive competition between consumption of carbohydrate to satisfy the energetic and precursor requirements for growth and wasteful consumption by uncoupled respiration. By consuming all available storage carbohydrate during the first few h of darkness, the mutant may deprive itself of the carbon required to maintain biosyntheses throughout the night period. The demand for substrate to support maintenance respiration during the latter phase of the dark period may actually stimulate catabolic destruction of recently synthesized macromolecules with the result that growth is severely impeded. Thus, starch may be important not only as a nonosmotic form of reserve carbohydrate, but also as a metabolically inactive reserve. By controlling the availability of respiratory substrate, the mechanisms regulating the activity of enzymes required for starch hydrolysis may, therefore, be an indirect but important determinant of respiratory efficiency.

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